Growth of Northwest Iberian juvenile hake estimated by combining sagittal and transversal otolith microstructure analyses

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Abstract

Daily growth of Atlantic juvenile hake from Northwest Iberia has been estimated employing a new approach combining analyses of transversal and sagittal sections of the otoliths along the ventral radius. Age of juvenile hake ranging from 3-25 cm collected during a spring 2002 survey was estimated. Somatic growth followed a power fit: Fish size (TL)= 3,3254*age ^{0,7336} (r^2=0,87, p<0.001, n=76), yielding an average individual growth rate of 0.66 mm/day (± 0.06). The growth model indicates that after a year's life a juvenile can reach 25 cm. Otolith ventral radius ranged from 401 to

1842µm and daily increments were between 104 and 387. Fish growth and otolith growth were closely related (r^2=0.92 $p<0.001$, n=76). These first results of daily growth rates for the Southern stock corroborate the fast growth hypothesis of this species. The evolution of increment widths from hatch dates onwards reveals important seasonal growth peaks during July-August and October-November. A comparison with prior data and discussion is also presented in the light of recent work on hake juveniles and tagging-recapture experiences.

Keywords: Juvenile hake, otolith microstructure, daily growth, seasonal variation, NW Iberian waters.

Introduction

European hake (*Merluccius merluccius*) is a demersal finfish inhabiting Atlantic and Mediterranean waters. It is widely distributed in the Northeast Atlantic from Norway to Mauritania, being more abundant from the British Isles to the south of Spain (Casey and Pereiro, 1995). It is mainly found between 50 to 370 m depth though its distributional bathymetric range is from 30 to 1000 m (Dominguez-Petit, 2007). In the Western European fisheries hake is one of the most valuable and heavily exploited demersal species. In recent years the status of European hake stocks (Northern and Southern) has been cause for concern, particularly the Southern stock that is considered to be outside safe biological limits (ICES, 2007). However, the biology of hake is insufficiently known to make accurate predictions, and in particular little is known about growth, although such knowledge is critical for accurate stock assessment.

European hake is a batch spawner (Murúa and Motos, 2006) with an indeterminate fecundity. In Iberian waters it has a protracted spawning season that covers the whole year, though most spawning activity occurs from December to July with, depending on the year, a main peak between January and March and a secondary peak in June-July (Dominguez-Petit, 2007). On the Galician and Cantabrian continental shelves there are important nursery areas located where juveniles measuring 8-14 cm long have been found from September to November (Sanchez and Gil, 1995, 2000).

The importance of growth prediction is reflected in the fast *versus* slow hake growth debate that has been ongoing since the 1930s (Hickling 1933; Belloc, 1935). However recent tagging experiments (de Pontual *et al.,* 2003, 2006; Piñeiro *et al.*, 2007), have provided direct evidence supporting the fast-growth hypothesis, as anticipated by Belloc (1935) and Bagenal (1954), which has implications on the management of the resource (Bertignac and de Pontual, 2007).

Tagging small juvenile fish is difficult and has not been successful yet because of the high natural mortality rate that occurs during early life stages. Therefore, an alternative approach based on otolith microstructure analysis was developed to analyze growth during the first year of life. Otolith incremental patterns have been validated for European hake at daily level, directly in the Bay of Biscay (de Pontual *et al.* unpublished data); indirectly in the Adriatic Sea (Arneri and Morales-Nin, 2000) and from a larval rearing experiment in Norway (Morales-Nin *et al.*, 2005).

Growth studies on juvenile hake have been carried out in different areas of the Mediterranean Sea (Morales-Nin and Aldebert, 1997; Morales-Nin *et al.,* 1998; Arneri and Morales Nin, 2000; Morales-Nin and Moranta, 2004; Belcari *et al.,* 2006) and on the northern stock in Atlantic waters (Kacher and Amara, 2005). Nevertheless, growth estimations are still missing for hake of the so-called southern stock

The purpose of this study is to estimate, for the first time, the growth rate of juvenile hake from northwestern Iberian waters which are home to an important nursery ground (Southern stock, ICES Divisions VIIIc and IXa). The study is based on age estimates from microincrement analyses of saggital and transversal otolith sections. Seasonal growth is analyzed to improve the understanding of growth variability over time at the juvenile life stage. The results obtained are compared with growth models reported by other authors and discussed in the light of recent work carried out on juvenile of hake and tag-recapture experiments.

Material and Methods

Juvenile hake were collected during a bottom trawl survey carried out in Spanish Atlantic waters (Fig. 1) from the 22nd to 30th of April 2002 on board the R/V "Cornide de Saavedra". The sampling scheme was randomly stratified according to depth and geographical criteria (Sanchez *et al.,* 1994, Sanchez and Gil, 2000). Hauls were performed during daylight hours at a depth range of 40 - 325 m. Sex, catch date and total length (± 1 mm) of fish were recorded on board, while otoliths (sagittae) were carefully removed from five specimens by length class and stored dry in vials for subsequent analysis in the laboratory. The terminology used for otolith sections is based

on the glossary edited by Panfili *et al.,* (2002).

A total of 157 pairs of otoliths were prepared from individuals between 3 and 25 cm (TL) long. Otoliths were embedded in polyester resin. Thin transversal sections (TS) were obtained from the right otoliths (N = 108) using an ISOMET saw machine. Sections were ground with sandpaper between 400 and 1200 μm and polished with a graded series of aluminium oxide of 3, 1 and 0.3 μm until thin slides with clear microincrement sequence along the ventral axis were obtained. The left otoliths (N = 49) were ground equally on both sides along sagittal plane (SS) until the central zone (CZ) increments were totally readable from the nucleus to the edge of accessory growth centers.

Age interpretation was carried out using a light microscope connected to a video camera that projects a live image of the otolith using the Image Analysis System OTO v3 software designed by Andersen and Moksness (1988). Increments were counted and increment widths measured at magnifications from X250 to X1000 where the greatest magnification corresponded to the core region (SS).

On TS, counts and measures were made along the ventral axis between the edge of the central opaque zone (COZ) and the otolith edge (Fig. 2). This otolith axis has been used for decades for macrostructural interpretation (Piñeiro and Sainza, 2003) and has been therefore chosen as the reference axis to undertake daily increment counts. To ensure that the same axis was used in both sections, SS counting was done throughout the ventral axis from the first discernible increment to the boundary with the accessory contiguous growth center. A linear relationship between the number of increments and

the radius of CZ was obtained from the SS. This relationship was applied to estimate the number of increments within COZ on TS. Thus, the age estimation was a 3-step process: 1) counting increments on TS from the edge of the central opaque zone to the otolith margin (Fig. 2c), 2) estimating the number of microincrements deposited in the CZ from the above relationship and 3) adding both estimates to obtain the total age. To assess age estimation errors the average percentage of error (APE) (Beamish and Fournier, 1981) and CV were calculated for the two readings made on TS.

Assuming that no age differences occur between right and left otoliths, the growth through the life cycle of juvenile hake was fitted to a power function. Individual growth increments of each larva were estimated from the derivative of the power function of growth and corrected using residuals of estimated to observed sizes as described in Ramirez *et al.* (2001).

Changes in increment width under transmission light microscopy of transversal sections were examined in relation to the presence of translucent and opaque zones in order to understand the seasonal growth variation pattern.

Results

A total of 76 transversal and 27 sagittal otolith sections could be read, while the rest were discarded due to over-polishing or an imprecise increment pattern (Table 1). The fish and otolith size, number of increments and the total radius recorded are summarized in Table 2. The saggital and transversal sections have a different appearance. The central region of SS is characterized by a core surrounded by accessory growth centers,

with individual increments being almost contiguous across the growth zones and corresponding to different points of nucleation (Fig. 2a). This complex structure appears as an opaque zone (COZ) on TS with poorly defined increments difficult to interpret (Fig. 2 b). From the COZ edge, increments tend to be wider and regularly spaced on the ventral axis (Fig. 2b, c, d).

Sagittal section

The core and accessory growth centers were completely formed in individuals at a minimum size of 3 cm TL, (Fig. 2a). Radius measurements along the ventral axis to the accessory growth centers were highly variable due to its irregularly shaped border. Thus, radius measurements to the accessory growth centers were independent of fish size.

The number of increments in the CZ ranged from 38 to 69, with a mean value of 51 (± 8.0) and a radius range of 89-212μm with a mean value of 141μm (± 31.6) (Table 2). Increment counts started from a first check observed at 24 μm (±3.9), presumably corresponding to a "first feeding check" (FFC). From this check onwards visible increments appeared with a progressively wider pattern (1-2 μm) from the FFC to the edge of accessory growth centers, with a mean value of 3 μm (± 0.9) and reaching a maximum value of 4μm at 40 increments (Fig. 3). From this point increment widths decrease gradually to 3 μm.

The ventral radius of CZ vs increment counts showed strong relationships both by linear and power functions fixing an intercept at 16.3μm as hatch check reported by Palomera

et al. (2005) (Fig. 4). Nevertheless, the power function has been considered more adequate to explain the early life otolith growth of this species (Radius=16,3+0,653978*age ^{1,33394}; R^2= 0,734).

Transversal section

The radius of ventral axis in TS ranged from 400-1842µm with a mean value of 1211µm (± 367.8) (Table 2). From the COZ edge, TS showed on the ventral axis, clear increment sequence with an average width of 6 µm (±1.6). Sequences of wide and narrow increment widths viewed under transmitted light at small magnification appear as translucent and opaque bands (see Fig. 2c). The number of increments observed from the COZ to the otolith edge ranged from 46 to 331. The APE and CV obtained from the readings were 4.5 and 1.3, respectively.

Fish growth and otolith growth are linearly correlated as indicated by the relationship between otolith ventral radius in the TS and fish total length ($y = 0.1035x + 50.019$; $r^2=0.92$, $p<0.001$; $n=76$). This allows fish size to be inferred from otolith size.

Growth model

The sum of estimates of SS and TS sections provide age estimates of the fish considered in this study. The estimated age-length relationship was best explained by a power fit whose origin intercepts at 0, which would lead to a juvenile size of 25.2 cm at one year (Fig. 5). No significant difference was observed when introducing a biological intercept

at 2.5 mm, size at hatch according to Palomera *et al.,* (2005). Individual growth increments show a significant decreasing trend with age (Fig. 6), which overall averaged is 0.66 mm/day (± 0.06).

Seasonal growth

Dating increments backwards from the most recent that coincide with capture date to the COZ area, increment width ostensibly varied with time (Fig. 7), corresponding to seasonal growth pattern. Two seasonal peaks of high growth are observed corresponding to July-August and October-November, when increment widths are greater than 6 μm. During the months of March-April and September, increment widths decrease substantially to widths measuring around 4 μm. Therefore, juvenile growth shows a seasonal trend where growth slows down in spring and early autumn (width<6 μm) and increases in summer and winter.

Discussion

To understand the process of growth through the juvenile life cycle it was considered important to gain insight of increment formation and deposition in early larval stages. Since larval growth studies on European hake are at present rather scarce due to their usually low catch of larvae by plankton gear (Palomera *et al.*, 2005; Alvarez and Cotano, 2005) on European hake from Mediterranean and Atlantic waters, this study allows us to infer larval growth pattern from the interpretation of juvenile otoliths.

Larval otoliths (sagittae) are disc shaped and, as they evolve over time, become increasingly asymmetrical and gain complexity with the formation of accessory growth centers until they reach a point at which age estimation of juveniles is difficult. The approach used in this study attempts to overcome this issue by combining age estimation from saggital and transversal planes to estimate the age of an individual.

Generally, it is assumed that increment formation in hake starts at hatching (Wright *et al.,* 2002,). The studies of Palomera *et al.* (2005) and Alvarez and Cotano (2005) describe a dark spot in the center of the sagitta forming a clear check around it that they attribute to a hatch check at a distance of 16.3 and 15.7μm, respectively. These authors observe a number of intermediate increments before the next check formation (4-6: Alvarez and Cotano, 2005; 4-5: Palomera *et al*., 2005), which presumably corresponds to the FFC, practically coincident with Bjelland and Skiftesvik (2006) who found that hake larvae start first feeding at 6 days after hatch from a larval rearing experiment. The first discernible increment observed on our SS was found at a mean distance of 24μm (± 3.9) from the core. From the relationship of CZ radius and SS increment counts in which 16.3μm is assumed as hatch check, after 6 days we would find the FFC at 23.5μm, in agreement with our assumed FFC.

Formation of the CZ and accessory growth centers are attributed to the pelagic life of the larval stage of hake and recruitment to the bottom (Morales-Nin and Aldebert, 1997; Arneri and Morales-Nin, 2000). The pelagic phase estimates from our observations (51± 8 days) are within the temporal range found by other studies (see Table 3), such as the work of Belcari *et al,* (2006), but slightly lower than that reported by Morales-Nin and Moranta (2004) on Mediterranean hake, and greater than that reported for Atlantic hake

by Katcher and Amara (2005). These differences may be due to either the different methodological approaches and/or to site dependent growth characteristics of hake living in different environmental conditions.

Larval otolith analyses also show differences in maximum age estimates of hake larvae between the Atlantic and Mediterranean (Alvarez and Cotano, 2005; Palomera *et al*. 2005), but these studies did not consider the whole early life history corresponding to CZ formation.

The TS used to account for the juvenile phase presented particularly clear sequence of microincrements (Fig. 2d) which at a macroscopic scale constituted successive wide opaque zones (OZ) and narrower translucent zones (TZ). The latter corresponds to either seasonal growth structures or fish specific responses to endogenous or environmental factors (Courbin *et al*., 2007).

This plane of sectioning was chosen in this study because sagittal and frontal sections did not allow accounting for the entire fish life (from the core to the edge) due to curvilenar growth of hake otoliths in respectively the distal-proximal and antero-posterior axis. The choice of a common interpretation axis between SS and TS (Fig. 2) allowed a complete reconstruction of the life history on the assumption of left-right symmetry generally fulfilled except in flatfish and catfish (Wright et al., 2002).

The measurement trajectory allows measuring the overall otolith ventral radius and estimating the number of increments, while the relationship between the ventral radius

of the TS is strongly correlated with fish length (R^2=0.92, p<0.001) and therefore allows a reliable estimation of fish size from otolith size.

Juvenile hake size showed clear exponential growth with the approach undertaken in this study. The relationship is supported by a strong relationship between otolith size, measured by the ventral radius, and daily increment counts which were highly correlated with linear and power fits. However, we consider that the power model provides a better description of otolith growth (r^2 = 0.92; p<0.001).

Averaged individual growth rates (0.66 mm/day ± 0.06) were high and the growth model indicates that at one year juvenile hake may attain 25.2 cm. These values are higher than most of the reported ones (Table 3), although they are in line with recent estimations by Morales-Nin *et al.* (2005), and Kacher and Amara (2005) which defend the fast-growth hypothesis. Furthermore, recent tag-recapture experiments provided direct evidence for the fast growth hypothesis (de Pontual *et al.*, 2006; Piñeiro *et al.*, 2007), showing that NE Atlantic hake (*M. merluccius)* may reach 25 cm TL at the end of the first year, instead of the 20 cm TL estimated from an internationally agreed age estimation method (Piñeiro and Saínza, 2003).

Growth studies based on otolith microstructure analysis have produced great discrepancies in the estimated growth rates in the juvenile phase and consequently on the size attained at the end of the first year of life. The highest daily growth rates (0.71-0.74mm/day) were found in Atlantic hake (Kacher and Amara, 2005) which would yield a juvenile TL of 23.8 cm at the end of a first year, while lower estimates have been obtained in the Mediterranean (see Table 3). These differences may be due to various

factors such as geographical locations, period of sampling and methodological approaches.

The method employed in this study allows a unidirectional linear measurement path for daily age interpretation, as recommended by Campana (1992). The main difficulty in age estimation is found in the transition area between the CZ and the prisms of accessory growth centres.

Analysis of otolith microstructure of the ventral axis indicated a variation of increment widths showing seasonal growth variations. There is a decrease in growth in spring and to a lesser extent in early autumn (width<6 μm), while there are more stable growth rates in winter and summer. Atlantic hake therefore follows a series of pulses of varying growth intensity throughout the year as also reported for Mediterranean hake (Morales and Moranta, 2004) and even undergoing a series of growth decreases during their first year of life in the Atlantic and Mediterranean hake (Piñeiro and Pereiro, 1993; Morales-Nin and Aldebert, 1997). The microstructural examination of increment width variations with time of this study confirms hake growth variability.

In conclusion, we applied a novel approach involving TS and SS microstructural analysis to estimate the growth of juvenile hake. The proposed approach will be useful for the comparison of structural patterns at micro and macroscopic scales. Such analyses are required for a better understanding of the typology of hake otolith macrostructure in relation to the biological meaning and mechanisms (endogenous and environmental) that control increment deposition. This approach also provides a new framework for the

development of an alternative method of age estimation of this species as the current one has been shown to be inaccurate (de Pontual *et al.,* 2006).

Our study indicates that, during their first year of life, hake grow at a faster rate than commonly accepted. Estimation of hake size at one year is in agreement with the hake growth model derived from tag-recapture data in both hake stocks (de Pontual *et al.*, 2006; Piñeiro *et al.*, 2007).

Such results are of real interest as underestimation of growth has implications stock assessment and management. As has been demonstrated for hake northern stock (Bertignac and Pontual, 2007), bias in estimating age affects the absolute levels of fishing mortality and stock biomass estimates, and also impacts on the predicted trend in SSB indicating that the stock may be more reactive to any change such as for instance fishing level, which affects medium and long term forecasts.

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Table 1

TL (cm)	SS	TS
<5	1	-
5-10	1	3
11 –15	8	27
16- 20	14	26
21 –25	3	20
Total	27	76

Table 1 .- Number of sagittal (SS) and transversal section (TS) otoliths analyzed by fish length class.

Table 2

SS	Fish Length (TL; mm)	First ring (µm)	Radius CZ (µm)	Increment width CZ (µm)	Daily Increments CZ
Mean	163	24	141	3	51
range	32-229	15-31	89.1-212	1-4	38-69
SD	41.7	3.9	31.6	0.9	8.0

TS	Fish Length (TL; mm)	Total VR (µm)	VR of COZ (µm)	VR without COZ (µm)	Daily Increm. without COZ	Total Daily Increm.
Mean	175	1211	142	1069	173	224
range	93-256	400-1842	76-208	230-1707	46-331	104-387
SD	39.7	367.8	31.9	371.2	66.9	65.9

Table 2.- Summary of statistics of data measurements obtained from the analysis of sagittal section (SS) and transversal section (TS) otoliths: fish length, first ring observed, radius of CZ (µm), increment width of CZ (µm), daily increments of CZ, ventral radius (VR, µm) of COZ, total ventral radius, daily increments without COZ and total daily increments.

Table 3

Table 3. Summary table of daily growth data obtained by different authors in different areas: Growth rate (GR), Central zone (CZ) and its daily growth increments (DGI), mean length at the first year (ML), otoliths sampled and fish length (FL) range and geographical area.

Author	GR	CZ size ø= diameter ®=radius (μm)	N. (mean± SD) or range number of DGI into the CZ	ML at first year of life (TL, cm)	Otoliths sampled and FL range	Area
Morales Nin and Aldebert, 1997	1.15 cm/month	162± 43 ø	(43.9± 19.7)	16	81 10.5-20.7 cm (TL)	Gulf of Lions
Arneri and Morales-Nin , 2000	1.1-1.6 cm/month	-	-	15	145 1.6- 16.4 cm (TL)	Central Adriatic
Morales Nin and Moranta, 2004	1.2 -2.5 cm/month	-	63	-	153 2.5-25 cm (TL)	Mediterranean Sea
Kacher and Amara, 2005	0.72-0.74 mm/day	-	(39± 7, n=13)	23.8	107 6-22 cm (TL)	Bay of Biscay and Celtic Sea
Palomera *et al.,* 2005	0.15-0.19 mm/day	(18.9-221.1) ø	6-26	-	71 2.5-9.1mm (SL)	Mediterranean Sea
Alvarez and Cotano, 2005	0.15-0.17 mm/day	-	3-40	-	40 2-12 mm (SL)	Bay of Biscay
Morales Nin *et al.*, 2005	1.8 cm/month	461.28 ø	64	23.7	1 13.5 cm (TL)	Rearing conditions
Belcari *et al.,* 2006	1.3 - 1.7cm/month	-	(52± 2)	18.3	579 4 – 20 cm (TL)	Tyrrhenian Sea
This study	0.66±0.06 mm/day (mean± sd,).	(89-212) ®	(51.2±8, n=27)	25.2	103 3-25cm (TL)	NW Spanish Atlantic

Figure 1

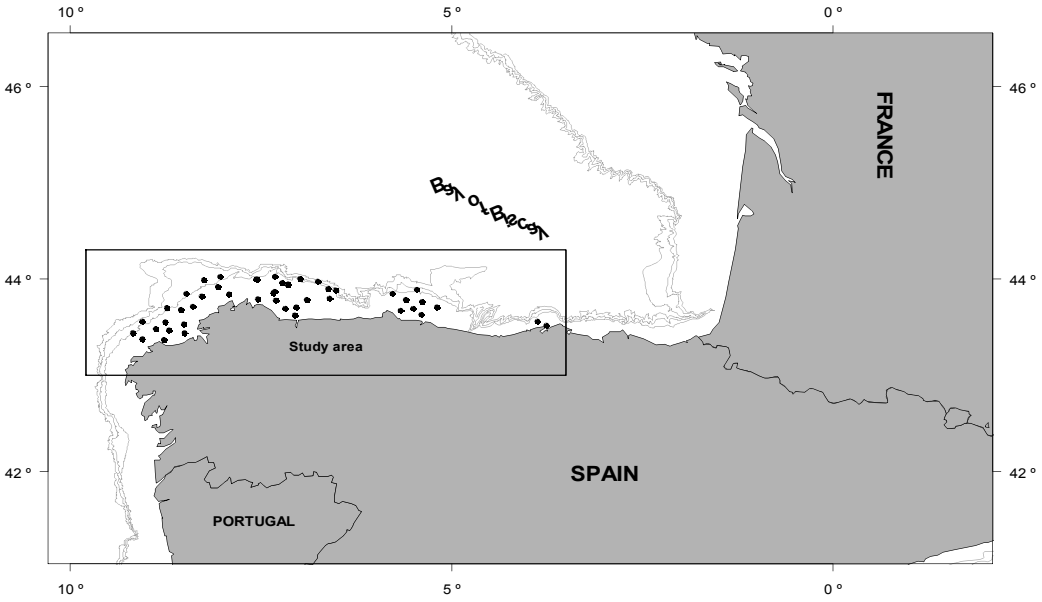

Figure 1.- Map showing the survey area and the locations from which otoliths samples were obtained.

Figure 2

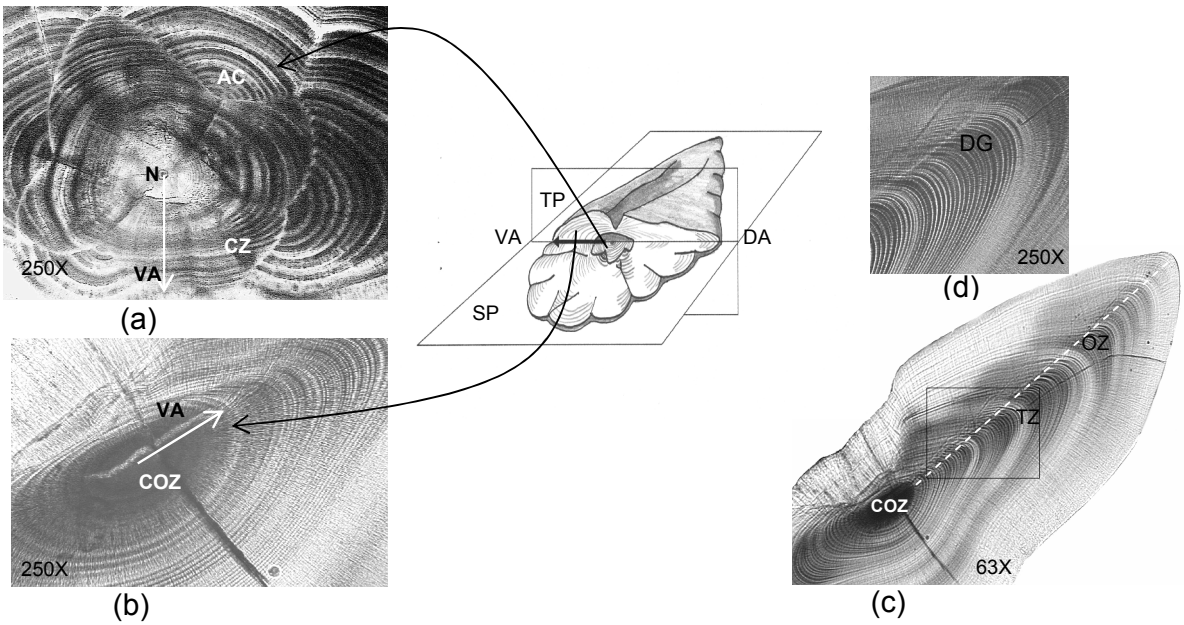

Figure 2. Drawing showing the view of the two sectioned planes of a hake otolith: saggital (SP) and transversal planes (TP) and the dorso-ventral axis (DA, VA). The saggital section, shows the CZ with an arrow indicating the ventral axis used for counting (VA), nucleus (N), and accessory centers (AC) (a). The transversal section shows the central opaque zone (COZ) where increments are not visible (b) and the view of the ventral axis chosen for radius measurement and growth increments along with changes in increment width (c). Detail of a sequence of growth increments (DG) showing translucent (TZ) and opaque bands (OZ) (d).

Figure 3

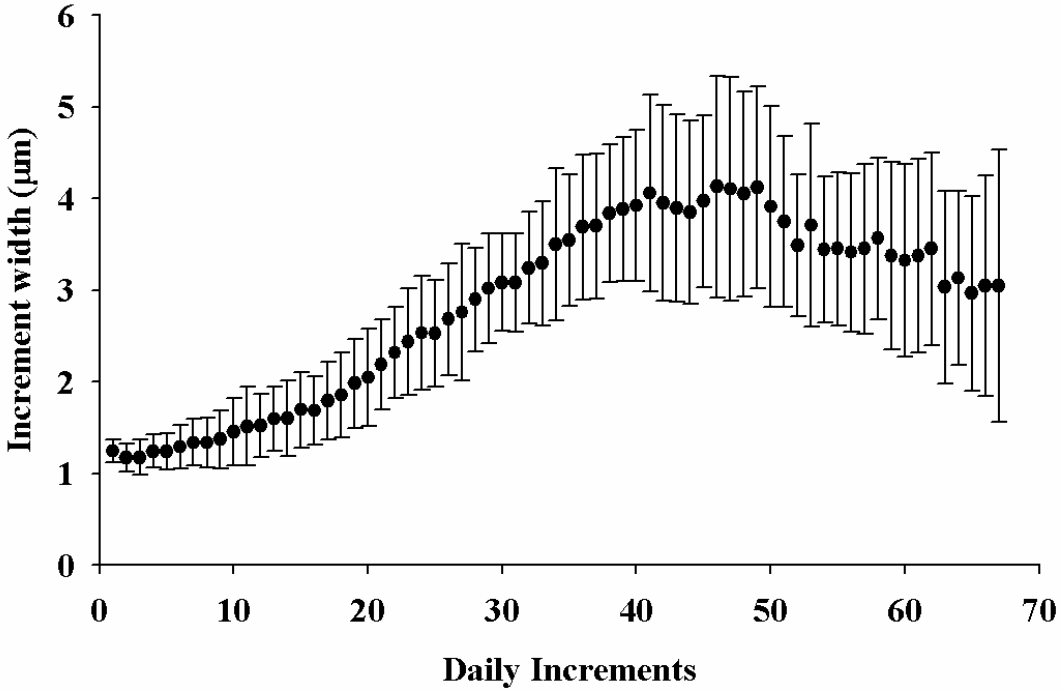

Figure 3.- Increments width (mean ± SD) within the CZ area of sagittal sections.

Figure 4

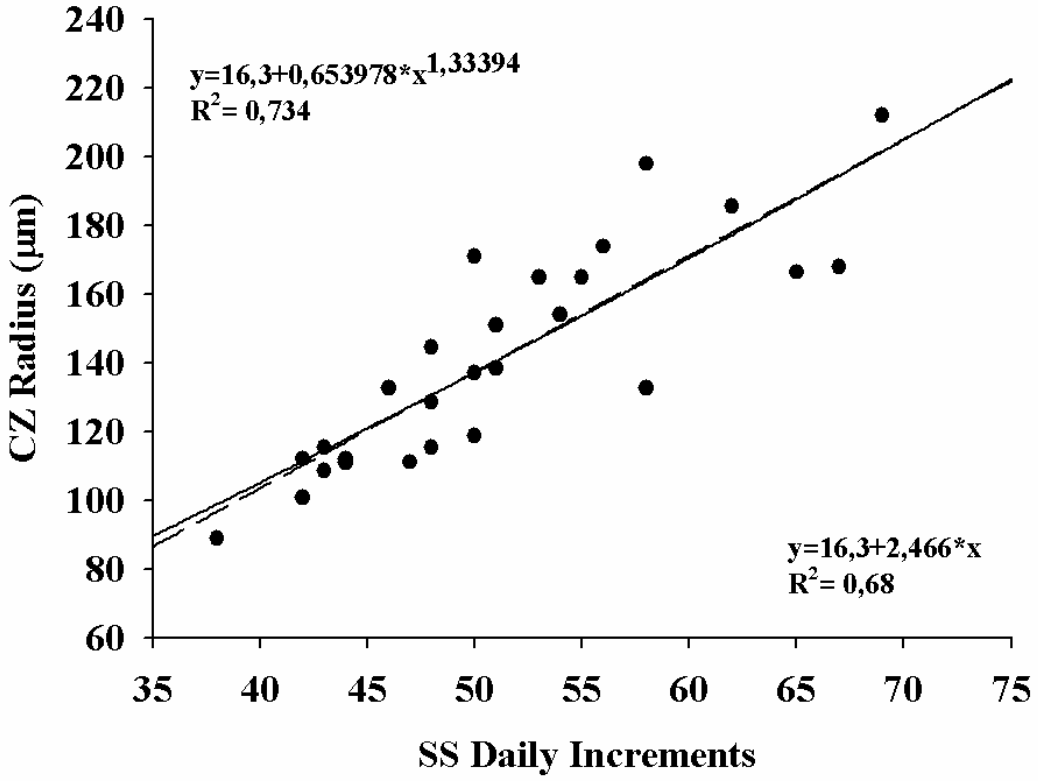

Figure 4.- Relationships (Power and Linear) between CZ radius and CZ daily increments of the sagittal section.

Figure 5

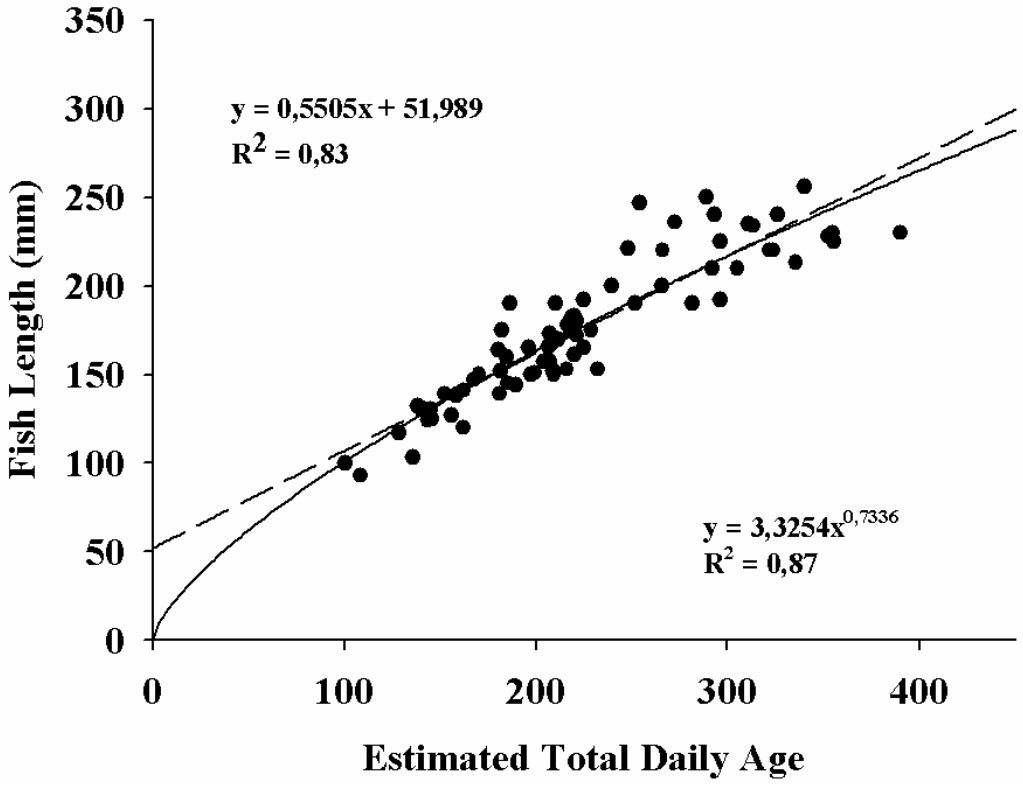

Figure 5.- Growth models (Power and Linear) of hake juveniles using estimated daily increments counts from TS and SS otoliths.

Figure 6

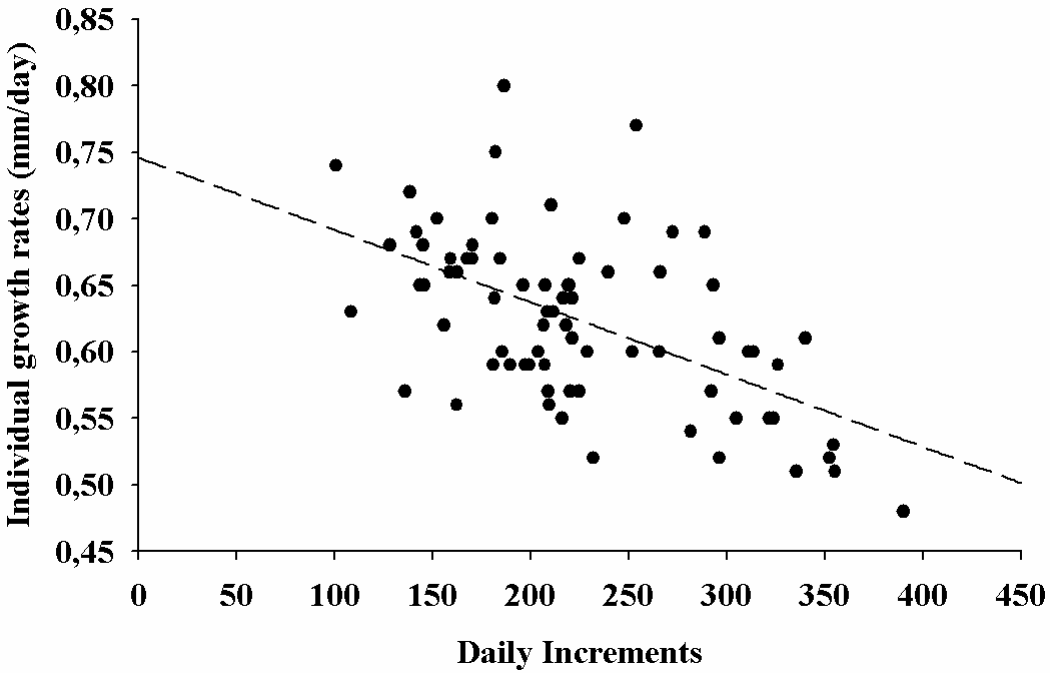

Figure 6.- Individual growth rates of hake juveniles by age.

Figure 7

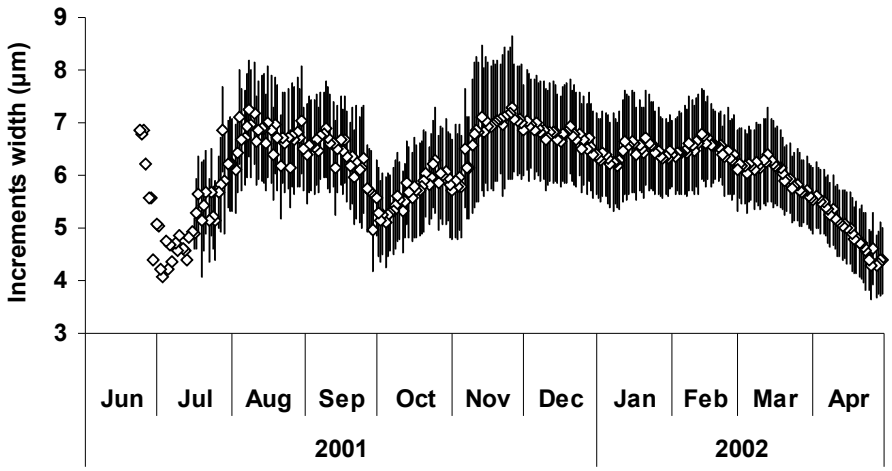

Figure 7.- Increments (mean widths) from individual capture date (most recent increment) to the COZ area of the transversal section.